Effect of thyroid hormone on somatomedin-C release from perfused rat liver

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Summary. The effect of thyroid hormone on plasma somatomedin-C (SmC) level and on SmC release from perfused rat liver was investigated. Plasma SmC levels and liver tissue SmC were significantly increased in thyroxine-treated rats. Physiological doses of triiodothyronine increased SmC release and SmC concentration in the perfused rat liver. These results indicate that thyroid hormone directly enhances the synthesis and release of SmC in the rat.
Key words. Thyroid hormone; somatomedin-C; perfused rat liver.

Radioimmunoassayable somatomedin-C (SmC)/insulin-like growth factor I levels are low in hypothyroidism and rise following thyroxine administration[1,2]. It is known that triiodothyronine (T_3) is able to directly stimulate the incorporation of sulphate into costal cartilage from dwarf mice in the absence of growth hormone, and also the production of SmC by cultured fetal hypothalamic cells in serum-free medium[3-5]. These results indicate that high levels of serum thyroid hormone would normally induce an increase in SmC secretion not necessarily mediated through growth hormone production. Phillips et al.[6] have reported that thyroid hormone appears to act independently from hGH on the release of SmC by the liver. To further clarify the relationship between thyroid hormones and SmC, we studied the effect of thyroid hormone administration on the plasma level of SmC and the effect of thyroid hormone on SmC release from the perfused liver in the rat.

Materials and methods. Dextran T-70 was purchased from Green Cross Co., Tokyo. Bovine serum albumin (BSA, fraction V), L-thyroxine, and 3,5,3'-triiodothyronine were obtained from Sigma Chemical, St. Louis, MO. L-thyroxine was dissolved in a small volume of 0.01 N NaOH and adjusted to a concentration of 50 µg/ml with saline.

Animals. Male Wistar albino rats weighing about 100 g were used. The rats were divided into two groups, control and experimental. Experimental rats were treated with subcutaneous T_4 (50 µg/kg/day) for 7 days. The control rats received vehicle alone. After 7 days, blood was drawn from the femoral vein for plasma T_4, T_3 and SmC analysis in fed rats. The livers were removed and weighed, and immediately frozen.

Liver perfusion. A modification[7] of the method of Sugano et al.[8] was used for isolation and perfusion of the rat liver in situ. The fed rats were anesthetized with pentobarbital sodium (30 mg/kg i.p.), and the abdomen was opened through a midline incision. The intestines were then placed to the animal's left. The thin strands of connective tissue between the right lobe of the liver and the vena cava were cut and a loose ligature was placed around the inferior vena cava above the right renal vein, the superior mesenteric and celiac arteries, and the portal vein. The portal vein was then cannulated and the perfusion pump was started. The ligatures around the portal vein and the arteries were tied. Then the thorax was opened and an outflow cannula was inserted through the right atrium into the thoracic vena cava.

Finally, the ligature around the abdominal inferior vena cava was tied, thus closing the circuit. The liver was perfused without recirculation with a synthetic medium at a flow rate of 3.5 ml/g liver weight/min in situ. After perfusion, the liver was weighed and immediately frozen.

Perfusion medium. The perfusion medium consisted of a Krebs-Ringer bicarbonate buffer containing 0.5% BSA, 4.6% dextran T-70, 5.5 mM glucose and sufficient outdated human erythrocytes to give a hemoglobin of 2.5%. The T_3 concentration in the perfusate was 200 ng/dl in the present experiment.

Perfusion methods. The liver was perfused for 15 min with medium lacking T_3, and then the appropriate high concentration of T_3 solution was added through a side arm syringe to the basic perfusion medium by means of a constant infusion syringe (0.1 ml/min). The liver was perfused with medium containing T_3 (200 ng/dl) for 30 min. The venous effluent was collected every 5 min and immediately centrifuged. The medium was stored at −20 °C until the time of SmC assay. During perfusion, the medium and the liver were warmed and kept at 37–38 °C and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4. To examine possible nonspecific effects of vehicle infusion, appropriate vehicle was added through a side arm syringe and SmC release by livers was observed.

Measurements. T_4 and T_3 were measured by appropriate RIA[9,10]. The RIA for SmC was carried out on unextracted samples using a nonequilibrium technique according to the method of D'Ercole et al.[11].

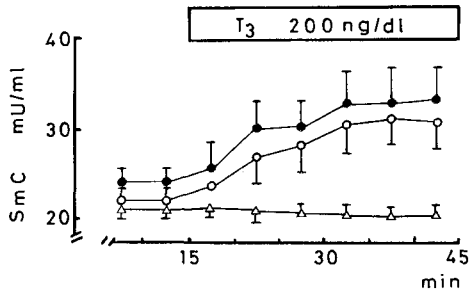
The standard is a lyophilized pool of plasma from normal adult human. Because normal adult rat serum has more SmC activity than human serum, the values reported for normal rat serum are relatively high. Intraassay and interassay coefficients of variation were 5% and 10% respectively.

Extraction of SmC from liver. SmC was extracted from the liver by the method of D'Ercole et al.[11]. In brief, each liver was homogenized and extracted with 1 M acetic acid, using 5 ml/g tissue. This procedure results in a final pH ranging from 3.6–4.2. Plasma somatomedins are liberated from their binding proteins by exposure to acid[12], and bioassayable somatomedin has been extracted from rat liver with acetic acid[13]. The extraction mixture was incubated on ice for 2 h and centrifuged (600 × g) for 10 min, and the supernatants were decanted. The pellet was reextracted once, and the supernatants were combined, frozen at −20 °C. The supernatants were lyophilized to dryness, and reconstituted with 0.05 M Tris buffer (pH 7.8) at a ratio of 2 ml of buffer to 1 g of original tissue. The extract was clarified by centrifugation at 1000 × g for 5 min. Extracts were then frozen at −20 °C

Body weight, liver weight, plasma levels of T_4, T_3 and SmC and liver tissue SmC

	Body weight (g)	Liver weight (g)	T_4 (µg/dl)	T_3 (ng/dl)	Somatomedin-C Plasma (U/ml)	Liver (U/liver)
Control (n = 7)	121 ± 5	5.6 ± 0.2	4.8 ± 0.5	104 ± 20	2.4 ± 0.2	8.3 ± 0.4
T_4-treated (n = 7)	111 ± 7*	4.8 ± 0.3**	18.0 ± 3.0***	359 ± 80***	3.3 ± 0.3***	10.3 ± 0.5***

means ± SD, * $p < 0.05$, ** $p < 0.02$, *** $p < 0.005$.



SmC release from perfused rat liver. The bars represent SD (n = 6); ○, liver of control rats perfused with T_3; ●, liver of T_4-treated rats perfused with T_3; △, liver of control rats perfused with vehicle; T_3 infusion significantly increased SmC concentration in the effluent from livers of control and T_4-treated rats.

and stored until the assay for SmC. Using this method, the efficiency of extraction of added SmC was 75 ± 5%.

Statistical analysis. Statistical differences were determined by Student's unpaired two-tailed t-test.

Results. Body weight, liver weight, plasma levels of T_4, T_3 and SmC and liver SmC. As shown in the table, body weight (111 ± 7 g, mean ± SD) and liver weight (4.8 ± 0.3 g) in T_4-treated rats were significantly lower than those (121 ± 5 g and 5.6 ± 0.3 g) in controls. Plasma levels of T_4 (18.0 ± 3.0 µg/dl), T_3 (359 ± 80 ng/dl), SmC (3.3 ± 0.3 U/ml) and liver tissue SmC (10.3 ± 0.5 U/liver) in T_4-treated rats were significantly higher than those (4.8 ± 0.5 µg/dl, 104 ± 20 ng/dl, 2.4 ± 0.2 U/ml and 8.3 ± 0.4 U/liver) in controls.

SmC release from perfused rat liver and liver tissue SmC. As shown in the figure, basal SmC release from perfused rat liver was 22–24 mU/ml. And SmC release was increased to 30–33 mU/ml by the addition of T_3 (200 ng/dl) to the perfusing medium. Control vehicle infusion through a side arm to the basic perfusion medium did not increase SmC concentration in the effluent.

Liver tissue SmC concentration in T_4-treated (6.2 ± 0.5 U/liver) and control rats (5.6 ± 0.5 U/liver) perfused with 200 ng/dl T_3 was significantly higher ($p < 0.005$) than that (3.3 ± 0.4 U/liver) in control rats perfused with control vehicle.

Discussion. In the present study, plasma SmC levels were significantly increased in T_4-treated rats. This result was in agreement with the report of Cavaliere et al.[14] that T_4 administration to normal man increased plasma SmC levels. Liver tissue SmC concentration was also significantly increased in T_4-treated rats. These observations indicate that thyroid hormone administration may increase SmC synthesis in the liver. According to previous reports[3–5], thyroid hormone may act independently from growth hormone on SmC synthesis and/or release. Therefore, in the present study, the direct effect of triiodothyronine on SmC release and synthesis in the liver was investigated. An addition of physiological doses of T_3 (200 ng/dl) significantly increased SmC release and SmC concentration in the perfused rat liver. The present results suggest that T_3 may directly enhance the release and synthesis of SmC in the rat liver. Increased plasma levels of SmC in T_4-treated rats may partly be due to increased release of SmC from the liver. The mechanism by which T_3 directly enhances the synthesis and release of SmC in the perfused liver remains to be elucidated.

In summary, we conclude that T_3 directly enhances the synthesis and release of SmC in the liver and increases the plasma level of SmC in the rat.

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The effect of homogenization temperature upon the apparent cellular compartmentalization of unoccupied estrogen receptor[1]

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Summary. Homogenization of rat uterus at elevated temperatures results in an increased nuclear localization of unoccupied estrogen receptor. This is a nonlinear effect which is accounted for by an increased population of KCl-resistant nuclear binding sites at the elevated homogenization temperatures.
Key words. Estrogen receptor; homogenization; temperature; nucleus; uterus.

Early investigations on the mechanism of action of estrogen in reproductive tissues suggested a two-step process of intracellular hormone binding and subsequent translocation of the hormone-receptor complex to the nucleus[2–4]. This proposed mechanism was based upon the observation that estrogen binding was primarily localized to the cytoplasmic